Journal of **BIOLOGICAL RESEARCHES**

ISSN: 08526834 | E-ISSN:2337-389X

Volume 24| No. 2| June| 2019

Original Article

Phytochemical Study On The Flower of *Alstonia macrophylla* Wall. ex G.Don (Apocynaceae) from Sumbawa Island, Indonesia

Putri Sri Andila*, Tri Warseno, I Putu Agus Hendra Wibawa, I Gede Tirta
Bali Botanical Garden, Indonesian Institute of Science, Bali, Indonesia

Abstract

Alstonia macrophylla Wall. ex G.Don is a native plant to South East Asia, belonging to Alstonia genus. This species has been reported to have numerous natural chemical compound which perfom multiple pharmacological and biological activities. The aim of this study was to investigated the phytochemical properties of the acetone extract of the flower of A*lstonia macrophylla* Wall. ex G.Don. This is very interesting because phytochemical properties of its flower had been never reported yet. *Alstonia macrophylla* was harvested from the Natural Forest of Punik, Batu Dulang Village, Batulanteh Subdistrict, Sumbawa Regency, West Nusa Tenggara, Indonesia on Mei 2015. Acetone flower extract of *A. macrophylla* was analyzed with a GC-MS method to determine the chemical components. Result of GC–MS chromatogram revealed that there were 30 identified components in this extract. The major compounds were Cycloartenol acetate (17.11 %); 5H-1-Pyrindine (12.44 %); Lupeyl acetate (10.12%); Oleic acid (6.08 %); Benzenesulfonic acid, 4-hydroxy- (4.25 %); p-n-Amylphenol (4.23 %); and 4-Methylindole (4.22 %). Here, We reported the first study of phytochemical properties of *A. macrophylla*. This study help to understand further detail the potential of bioactive compound of *A. macrophylla*.

Keywords: Acetone Extract, *Alstonia macrophylla*, Flower, GC-MS, Phytochemical

Received: 30 April 2019 Revised: 10 May 2019 Accepted: 25 June 2019

Introduction

Natural products are chemical properties derived from a living organism, such as bacteria, animal, and plant. In plant, natural product commonly is produced in the form of secondary metabolites. The secondary metabolite occurs in the various part of plants such as leaves, stem and root. The compound is an importhant material for plant for defencing and protection mechanisms from various infection. Interestingly, the secondary metabolite obtained from plant has various biological activities and contains drug-like properties. Due to this reason, for a long time, the research about discovering and developing new drug compounds from secondary metabolites have been an important resources to be studied (Yuan et. al., 2016). For example, Cragg et al. (2013) reported that approximately 54 % of anticancer drug have been isolated from plants, such as paclitaxel (terpene) from *Taxus baccata*, vinca (alkaloids) from *Catharanthus roseus* (Cragg et al., 2013) and extract of *Alstonia scholaris* (Ahmad et al, 2016).

Alstonia consists of 40-60 species belong to Apocynaceae family. This genus is distributed and native to tropical and subtropical regions in Central America, Australia, Polynesia, Africa, and South East Asia with central diversity in the malesian (Sidiyasa, 1998a). Several species of this genus have been identified to be useful for treatment in various human diseases, such as *A. macrophylla*, *A. scholaris*, *A. boonei*, and *A. congensis*. *A. macrophylla* is one species of this genus which is locally named Pule Batu in Ambon (Indonesia), Pulai penipu Bukit, sayongan, and pulai daun besar, in Malaysia, Batino in Philippines and Tung fa in Thailand (Cheenpracha et al., 2013, Prosea, 2016). This species is classified as 'Least Concern' according to the IUCN Red List (Sidiyasa, K. 1998) and native to South East Asia with distribution region covers from Sri Lanka, South East Asia, Nicobar Islands, and New Guinea (Prosea, 2016).

In many countries, *A. macrophylla* has been used in a different kind of traditional medication to treat several human ailments and diseases. Das *et al.* (2006) reported that local community in India use the *A. macrophylla* stem bark to medicate various types of human disease, such as urinary infection, stomachache, malaria fever, skin diseases, swelling, and bone fracture. They also use the root of *A. macrophylla* to treat a bone fracture (Verma et al., 2010) and the leaves and root to break a fever (Elanchezhian et al., 2007; Arora, 2010).

Other literature also indicates that the traditional community in the Little Andaman Island, India consume a decoction of leaves and stem bark of *A. macrophylla* for treatment some human diseases, among others: stomachache, a different type of skin infections and urinary tract infections (Bhargava, 1983). In the Philippines, the leaf of *A. macophylla* is traditionally used to treat sprains, bruises, and joints disorder. While its bark is used for the treatment of fever, exhaustion, irregular periods of menstrual cycle, malaria, hepatic disease, bloody diarrhoea, diabetes and worm infection (Wiart, 2006). This practice is almost similar to traditional medication in Thailand. Changwichit et al. (2011) reported that the local people in Thailand use the bark and leaf of *A. macrophylla* for curing some human disorder, such as

* Corresponding Author:
Putri Sri Andila
Bali Botanical Garden, Indonesian Institute of Science, Bali, Indonesia
Phone: +6285210065479 Fax: +623682033171
e-mail: putribot11@gmail.com

http://dx.doi.org/10.23869/bphjbr.24.2.20198

impotent, diarrhoea, cholera, dysentery, fever, irregular menstrual period, and as vulnerary agents for healing and treating wounds. In Indonesia, Lombok, the concentrated aqueous extract of leaves, roots or bark of the young tree of *A. macrophylla* is used to treat ulcers (Hadi and Bremner, 2001).

The recent studies in pharmacology showed that *A. macrophylla* had a different type of pharmacological and biological activities. Keawpradub et al. (1999) revealed that the methanol extract of the root bark of *A. macrophylla* had antimalaria activity. While the stem bark extracts of *A. macrophylla* exhibited antiprotozoal (Camacho et al., 2003) and antimicrobial activity (Chattopadhyay *et al*., 2001; Khyade and Vaikos, 2010). Previous literature also performed that the leaves extract of *A. macrophylla* had several pharmacology and biology potentials, such as antimicrobial (Chattopadhyay *et al*., 2001), antioxidant (Arunachalam et al., 2009), Anti-inflammatory (Arunachalam *et al*., 2002, Chattopadhyay *et al.*, 2005a), Antipyretic (Chattopadhyay et al., 2005b), central nervous system drug (Chattopadhyay et al., 2004), Antifertility (Chattopadhyay *et al.*, 2005c) and antidiabetic (Arai *et al.*, 2010).

Several phytochemical studies in the different organ of *A. macrophylla* have been reported in scientific journals, such as Khyade et al. (2014) published that in leaves extract of *A. macrophylla* contained a various chemical contents, as well as terpenoid, alkaloid, sterol, and flavonoid. But the studies about secondary metabolite compound from the flower of *A. macrophylla* have never been published yet. Therefore, this study aimed to collect more information on the chemical composition of the acetone extract of *A. macrophylla* flower from The Natural Forest of Punik, the village of Batudulang, Subdistrict of Batulanteh, Regency of Sumbawa, West Nusa Tenggara, Indonesia.The results of this study were expected to explore further detail information and potential in phytochemical contents in *A. macrophylla* flower.

Methods

Plant Materials and Sample preparation

The flowers of *A. macrophylla* were collected from the Natural Forest of Punik, in Batudulang village on Mei 2015. This village is located in the area of Batulanteh Regency, Sumbawa Island, West Nusa Tenggara Province, Indonesia with an altitude 965 meter above sea level and geographical point: S 08°35'.866" and E117°14'501" (Shown in Figure 1). The plant was identified by Mr Ida Bagus Ketut Arinasa, M.Si, a taxonomist of Bali Botanic Garden and the herbarium was saved in the herbarium of Tabanan Hortus Botanicus Baliense (THBB).

Extraction

One kilogram of fresh material of the *A. macrophylla* flowers were prepared and minced into small pieces (about 1 cm in size). Then, the material was dried until perfectly dry at room temperature and without direct sunlight. One hundred grams of the dried materials were extracted with acetone by maceration method (soaking for three days in acetone) and the suspension was obtained by filtering the acetone extract with the filter paper (Azwanida, 2015). This suspension was analyzed with a GC-MS method to identify the name of phytochemical compounds.

Figure 1. Location of the sampling sites of *Alstonia macrophylla*, in The Punik Natural Forest, Batu Dulang Village, Batulanteh Subdistrict, Sumbawa Regency, West Nusa Tenggara, Indonesia (Google Maps 2019).

GC-MS Analysis and Identification of the Compounds

The GC-MS analysis of the acetone extract of the *A. macrophylla* flowers was conducted using GC-MS equipment "Shimadzu GC-MS – QP2010". The chroma-to-graphic column was equipped with Rtx 5 ms, and capillary column 60.0 m x 25 mm with 0.25 µm thickness. In Gas Chromatography Setting, the helium UHP was used as carrier gas at a total flow rate of 46.5 mL/min, and the column flow rate of 0.85 mL/min. The total volume of sample injection was 1 µL and the temperature of injector was maintained at 280 ℃ (with split ration 1:50). The column oven temperature was arranged at 50 ℃ for ±5 min then raised to 280 ℃, with total running time 50 min and pressure 101.0 Kpa. While the mass spectrum (MS) was set up with the ion source temperature

200 ℃, interface temperature 280 ℃, and detector temperature: 280 ℃ (Andila et al., 2019).

Data Analysis

The relative concentration of each component in the GC-MS result was obtained by comparing the peak area to the total peak area. The identification of chemical compounds identity was conducted using WILEY7 database (Andila et al., 2019).

Results

The GC-MS analysis result of the acetone extract of the *A. macrophylla* flowers was shown in table 1 and the GC-MS chromatogram was performed in figure 2. In this study was obtained 30 identified chemical compounds with dominant components were Cycloartenol acetate (17.11 %); 5H-1-Pyrindine (12.44 %); Lupeyl acetate (10.12%); Oleic acid (6.08 %); Benzenesulfonic acid, 4-hydroxy- (4.25 %); p-n-Amylphenol (4.23 %); and 4-Methylindole (4.22 %).

Table 1. The result of GCMS analyses obtained from the acetone extract of the *A. maccrophylla* flower from The Punik Natural Forest, Batu Dulang Village, Batulanteh Subdistrict, Sumbawa Regency, West Nusa Tenggara, Indonesia

Peak	Compounds of the acetone extract of the *Alstonia macrophylla* flowers	R.Time	SI	Relative Conc. %
1	Benzenesulfonic acid, 4-hydroxy-	9.773	95	4.25
2	l-Limonene	10.132	94	2.46
3	n-(methyl-d2)-aniline	11.075	82	1.62
4	2,3-dihydro-benzofuran	12.744	82	1.24
5	Benzenepropanenitrile	12.858	88	0.75
6	Safrole	13.292	93	1.31
7	5h-1-pyrindine	13.487	83	12.44
8	1-Iodo-2-methylundecane	14.095	85	1.33
9	4-Methylindole	14.342	95	4.22
10	5-Methylquinoxaline	14.763	80	1.15
11	.beta.-Isodurylonitrile	15.127	73	1.93
12	2,3-Dimethylindole	15.258	91	1.20
13	Benzene,[(1-methylethylidene)cyclopropyl]-, (R)-	15.458	76	2.11
14	Lauric acid	15.628	85	1.77
15	Hexadecane	15.743	84	2.03
16	12-methyl-oxa-cyclododecan-2-one	15.921	67	1.97
17	tetrahydro-4-(2-methyl-1-propene-3-yl)-2h-pyran-2-one	16.245	83	1.65
18	Psoralene	17.060	63	3.20
19	Palmitonitrile	18.022	88	2.00
20	Oleic acid	18.444	92	6.08
21	1,4-diaza-2,5-dioxo-3-isobutyl bicyclo[4.3.0]nonane	18.642	89	1.49
22	10-methyleicosane	19.172	94	1.18
23	amide 16	19.839	91	1.69
24	Tetracosane	20.325	97	1.16
25	Oleoamide	21.031	83	0.69
26	p-n-Amylphenol	24.029	86	4.23
27	4,4,6a,6b,8a,11,11,14b-octamethyl-1,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12	28.378	87	3.21
28	Cycloartenol acetate	29.260	71	17.11
29	5.beta.-pregna	30.681	71	1.49
30	Lupeyl acetate	42.248	88	10.12
	Total			97.08

Note: R.Time: Retention Time, Relative Conc. %: , Relative Concentration of each detected compounds of a total compound, SI: Similarity Index

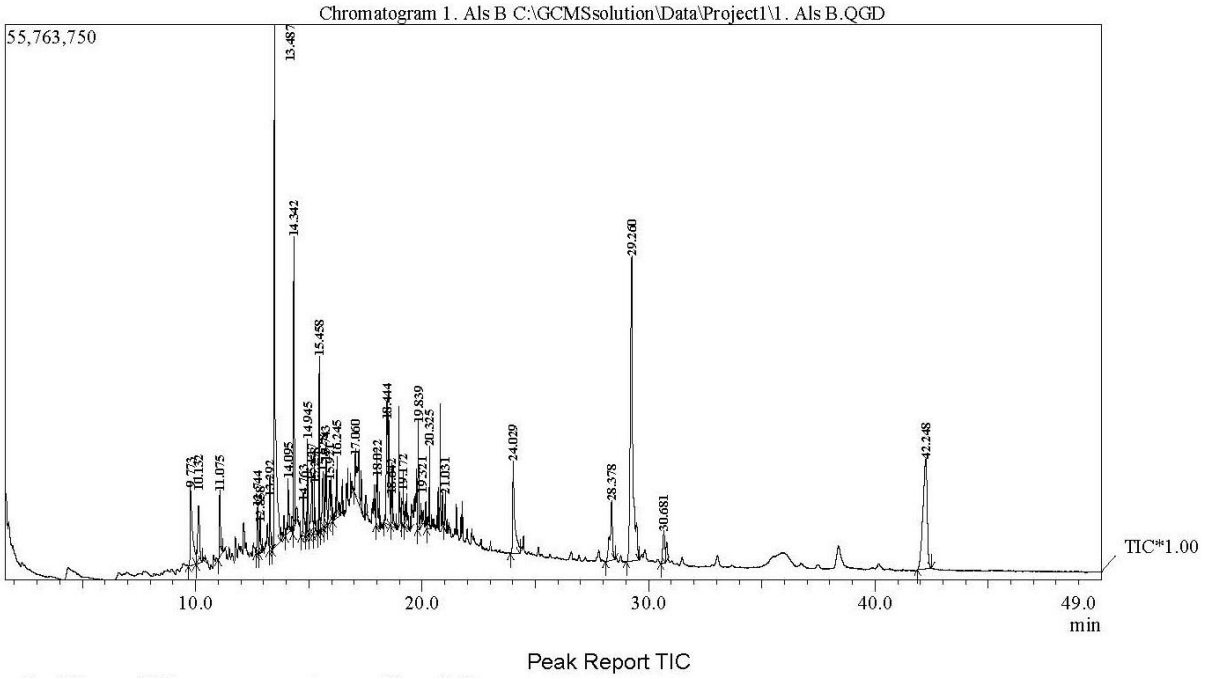

Figure 2. GC-MS chromatogram of the acetone extract of the *A. macrophylla* flower from The Punik Natural Forest, Batu Dulang Village, Batulanteh Subdistrict, Sumbawa Regency, West Nusa Tenggara, Indonesia

Discussion

The GC-MS analysis was used to determine the phytochemical properties in the acetone extract of the flower of *A. macophylla* obtained from the Natural forest of Punik, Batudulang village, Sumbawa regency, West Nusa Tenggara Province. The list of GC-MS analysis result was given in Table 1. and the chromatogram was perfomed in figure 2. The chromatogram showed 30 peaks in the total peak area, indicating the presence of 30 phytochemical contents. In this research, several terpenoid and fatty acid compounds were found, among others : Cycloartenol acetate 17,11 % (Triterpene), Lupeyl acetate 10.12% (Triterpene), l-Limonene 2.46% (Cyclic monoterpene), Lauric acid 1.77% (fatty acid), Oleic acid 6.08% (fatty acid), oleoamide 0.69% (fatty acid), and p-n-Amylphenol 4.23% (fatty acid).

As far as the author's knowledge, this study was the first report about phytochemical properties of *A. macrophylla* flower. By comparing with previous literature, the chemical properties identified from the flower extract of *A. macrophylla* were very different with the chemical contents from other part of *A. macrophylla*. For example, the previous study showed that *A. macrophylla* was abundant in alkaloids and nearly 70 alkaloid compounds were found in *A. macrophylla* (Khyade et al., 2014), but there were no alkaloid compounds that had been determined from its flower extract. A number of alkaloids had been found and isolated from leaves of *A. macrophylla*, among others : Nb-Demethylalstophylline oxindole (Atta-ur-Rahman et al., 1987), 16-Hydroxy-Nb-demethylalstophylline oxindole, Na-Methyl-1,2-dihydrostrictamine (Atta-ur-Rahman et al., 1988), Alstonamide, Alstoumerine, Demethoxyalstonamide (Atta-ur-Rahman et al., 1991), Alstofoline, Alstonoxine A, Alstonoxine B, N(1)-Demethylalsonisine, Isoalstonisine, Macrogentine, N(1)-Semethylalstonal (Kam and Choo, 2000), Alstohentine, Alstomicine, Alstomaline, Alstophyllal (8), 6-Oxoalstophylline; 10,11 Dimethoxynareline, 16-Hydroxyalstonisine, 16-Hydroxyalstonal, 16-Hydroxy-N(4) demethylalstophyllal oxindole, 6 Oxoalstophyllal (Kam and Choo, 2004), and Alstiphyllanines A–D (Hirasawa et al., 2009), Alstonal, Alstonerine, Alstiphyllanines E–I, Burnamine-17-O-30,40,50; trimethoxybenzoate, O-Deacetylpicraline, 10-Methoxy-N(1)-methylburnamine-17-O, veratrate, Picralina, Picrinine, Quaternine, Veratrate, Vincamedine, Vincamajine, and Vincamajine-17-O-trimethoxybenzoate (Arai et al., 2010). While the alkaloids found in stem bark of *A. macrophylla* were Macralstonine, Naresuanoside, Sweroside, Thungfaine (Changwichit et al., 2011), Alstoniaphylline A–C (Cheenpracha et al., 2013), Alstonerinal, 10-Methoxyaffinisine, 10-Methoxycathafoline (Kam et al., 1999), Angustimalal, N(1)-Demethylalstophylline 14, N(1)-Demethylalstophyllal, Macrocarpine A, Macrocarpine B, Macrocarpine C, Macrodasine A, Macrodasine B, N(4)-Methyl-N(4), 21, secotalpinine, Perhentinine (Kam et al., 2004), Lumutinines A–D, Perhentidines A–C (Lim et al., 2011), Alstonal, Nb-Demethylalstophyllal oxindole, Nb-Demethylalstophylline Talcarpine (Wong et al., 1996). In root bark of *A. macrophylla* was also contained some alkaloid compounds such as Alstomacrophylline, Alstomacroline, Alstonerine Alstophylline, 20-epi-Antirhine, Macrocarpamine, Villastonine N-oxide (Keawpradub and Houghton, 1997).

Besides Alkaloid, other groups of bioactive compound have also been found in leaves of *A. macrophylla*, among others: flavonoid (such as: Myricetin-30-rhamnoside-3-O-galactoside, Tricin-40-O-β-L-arabinoside, Vitexin) (Parveen et al., 2010), Pytosterols (such as β-sitosterol and β-sitosterol glucoside) and Triterpenoids (Ursolic acid, Pytosterols β-sitosterol and β-sitosterol glucoside) (Arunachalam et al., 2009).

Acknowledgments

We would like to express our special thank and appreciation to the essential oil research group at "Eka Karya" Botanical Garden- Bali for sponsoring this study (DIPA-2016). We also give our gratitude to Ida Bagus Ketut Arinasa, M.Si for his input in this research. We (Authors) also declare that Putri Sri Andila and Tri Warseno play a role as a main contributor in this study, while I Putu Agus Hendra Wibawa and I Gede Tirta are a member contributor.

References :

Ahmad, S., Ahmad, S., Ali, A., & Afzal, M. (2016). Anticarcinogenic and antimutagenic activity of Alstonia scholaris on the albino mice bone marrow cells and peripheral human lymphocyte culture against methyl methane sulfonate induced genotoxicity, 5(92), Advanced Biomedical Research. 1–11.

Andila, P. S., & Tirta, I. G. (2019). Distribution and Phytocomponent in the Ethanol Extract of Globba candida Gagnep . (Zingiberaceae) by GC-MS Analysis. Journal of Tropical Life Science 9(1), 43–51.

Arai, H., Hirasawa, Y., Rahman, A., Kusumawati, I., Cholies, N., Sato, S. & Morita, H. (2010). Alstiphyllanines E – H , picraline and ajmaline-type alkaloids from Alstonia macrophylla inhibiting sodium glucose cotransporter. Bioorganic & Medicinal Chemistry 18(6), 2152–2158.

Cheenpracha, S., Ritthiwigrom, T., & Laphookhieo, S. (2013). Alstoniaphyllines A − C , Unusual Nitrogenous Derivatives from the Bark of Alstonia macrophylla. Journal of Natural Products 76(4), 723–726.

Cragg, G. M., & Newman, D. J. (2013). A continuing source of novel drug leads. Biochimica et Biophysica Acta 1830(6), 3670–3695.

Das, S., Sheeja, T. E., & Mandal, A. B. (2006). Ethnomedicinal uses of certain plants from Bay Islands Ethnomedicinal uses of certain plants from Bay Islands. Indian Journal of Traditional Knowledge 5(2), 207–2011.

Elanchezhian, R, Senthil Kumar, R., Beena, S.J., & Suryanarayana, M.A. (2007). Ethnobotany of Shompens - A primitive tribe of Great Nicobar Island Ethnobotany of Shompens - a primitive tribe of Great Nicobar Island. Indian Journal of Traditional Knowledge 6(2), 342–345.

Guha Bakshi, D.N. & Sensarma, P., Pal, D.C. (1999). A Lexicon of Medicinal Plant in India. Vol. 2. Kolkata, India: Naya Prakash.

Hadi, S & Bremner, J.B. (2001). Initial studies on alkaloids from Lombok medicinal plants. Molecules 6(2), 117–129.

Hirasawa, Y., Arai, H., Zaima, K., Oktarina, R., Rahman, A., Ekasari, W., Widyawaruyanti, A., Indrayanto, G., Zaini, N.C. & Morita, H. (2009). Alstiphyllanines A - D , Indole Alkaloids from Alstonia macrophylla. Journal of Natural Product 72(2), 304–307.

Kam, T. & Choo, Y. M. (2004). New Indole Alkaloids from Alstonia macrophylla. Journal of Natural Product 64(7), 547–552.

Kam, T., Iek, I., Choo, Y., Silva, D., Silva, D., Silva, D., & Kam, M. A. (1999). Alkaloids from the stem-bark of Alstonia macrophylla. Phytochemistry 51(6), 839–844.

Keawpradub, N & Houghton, P. (1997). Indole alkaloids from Alstonia macrophylla. Phytochemistry 46(4), 1–5.

Keawpradub, N., Kirby, G. C., Steele, J. C. P., & Houghton, P. J. (1999). Antiplasmodial Activity of Extracts and Alkaloids of Three Alstonia Species from Thailand. Planta Medica 65(8), 690–694.

Khyade, M.S., Kasote, D.M. & Vaikos, N. P. (2014). Review: Alstonia scholaris (L.) R. Br. and Alstonia macrophylla Wall. ex G. Don: A Comparative Review on Traditional Uses, Phytochemistry and Pharmacology. Journal of Ethnopharmacology 153, 1–18.

Khyade, MS, Vaikos, N. (2010). Comparative phytochemical and antibacterial studies on the bark of Alstonia scholaris R. Br. and Alstonia macrophylla Wall. ex G. Don. Pharmaceutical Journal 1(4), 263–264.

Lim, S.H., Tan, S.J., Low, Y.Y. & Kam T.S. (2011). Lumutinines A_D, linearly fused macroline_macroline and macroline_sarpagine bisindoles from Alstonia macrophylla. Journal of Natural Product 74(12), 2556–2562.

Parveen, M., Khanam, Z., Akhtar, A. & Ahmad, S. M. (2010). A novel antimicrobial flavonoidic glycoside from the leaves of Alstonia macrophylla Wall ex A. DC (Apocynaceae). Chinese Chemical Letters 21(5), 593–595.

PROSEA. (2016). Alstonia macrophylla. Retrieved from https://uses.plantnetproject.org/e/index.php?title=Alstonia_macrophylla_(PROSEA)&oldid=218663.

Sidiyasa, K. (1998). Alstonia macrophylla. The IUCN Red List of Threatened Species 1998: e.T33194A9760084. Retrieved from http://dx.doi.org/10.2305/IUCN.UK.1998.RLTS.T33194A9760084.e

Sidiyasa, K. (1998). Taxonomy, Phylogeny, and Wood anatomy of Alstonia (Apocynaceae). Blumea. Supplement 11(1), 1–230.

Wiart, C. (2006). Medicinal Plants of the Asia-Pacific: Drugs for the Future. Singapore: World Scientific Publishing Co. Pte. Ltd.

Wong, W., Lim, P., & Chuah, C. (1996). Oxindole alkaloids from Alstonia macrophylla. Phytochemistry 41(1), 313–315.

Yuan, H., Ma, Q., Ye L. & Piao, G. (2016). The Traditional Medicine and Modern Medicine from Natural Products. Molecules 21(5), 1–18.